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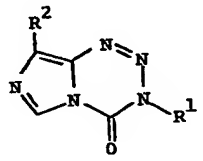
(54) **Tetrazine derivatives**

(57) [3*H*] Imidazo [5,1-d] - 1,2,3,5 - tetrazine - 4 - one derivatives of the formula:–



[wherein R^1 represents hydrogen, or an alkyl, alkenyl or alkynyl group containing up to 6 carbon atoms, each such group being unsubstituted or substituted by from one to three substituents selected from halogen atoms, alkoxy, alkylthio, alkylsulphinyl and alkylsulphonyl groups containing up to 4 carbon atoms, and optionally substituted phenyl groups, or R^1 represents a cycloalkyl group containing from 3 to 8 carbon atoms, and R^2 represents a carbamoyl group optionally N-substituted by one or two groups selected from alkyl and alkenyl groups containing up to 4 carbon atoms, and cycloalkyl groups containing 3 to 8 carbon atoms], are new therapeutically useful compounds possessing antineoplastic and immunomodulatory activity.

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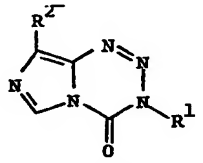
SPECIFICATION

Tetrazine derivatives

This invention relates to new [3*H*] - imidazo - [5,1 -d] - 1,2,3,5 - tetrazin - 4 - one derivatives, to processes for their preparation and to pharmaceutical compositions containing them.

The compounds of the present invention are the [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one derivatives of the general formula:–



I

wherein R^1 represents a hydrogen atom, or a straight- or branched-chain alkyl, alkenyl or alkynyl group containing up to 6 carbon atoms, each such group being unsubstituted or substituted by from one to three substituents selected from halogen (i.e. bromine, iodine or, preferably, chlorine or fluorine) atoms, straight- or branched-chain alkoxy, (e.g. methoxy), alkylthio, alkylsulphinyl and alkylsulphonyl groups containing up to 4 carbon atoms, and optionally substituted phenyl groups, or R^1 represents a cycloalkyl group, and R^2 represents a carbonyl group which may carry on the nitrogen atom one or two groups selected from straight- and branched-chain alkyl and alkenyl groups, each containing up to 4 carbon atoms, and cycloalkyl groups, e.g. a methylcarbamoyl or dimethylcarbamoyl group.

When the symbol R^1 represents an alkyl, alkenyl or alkynyl group substituted by two or three halogen atoms, the aforesaid halogen atoms may be the same or different. When the symbol R^1 represents an alkyl, alkenyl or alkynyl group substituted by one, two or three optionally substituted phenyl groups the optional substituents on the phenyl radical(s) may be selected from, for example, alkoxy and alkyl groups containing up to 4 carbon atoms (e.g. methoxy and/or methyl group(s)) and the nitro group; the symbol R^1 may represent, for example, a benzyl or *p*-methoxybenzyl group. Cycloalkyl groups within the definitions of symbols R^1 and R^2 contain 3 to 8, preferably 6, carbon atoms.

Preferred tetrazine derivatives of general formula I are those wherein R^1 represents a straight- or branched-chain alkyl group containing from 1 to 6 carbon atoms optionally substitued by one or two halogen (preferably chlorine, fluorine or bromine) atoms or by an alkoxy group containing 1 to 4 carbon atoms (preferably methoxy) or by a phenyl group (optionally substituted by one or two alkoxy groups containing from 1 to 4 carbon atoms, preferably methoxy), or R^1 repres nts an alkenyl group containing 2 to 6 carbon atoms (preferably allyl) or a cyclohexyl group.

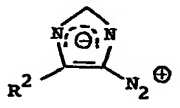
More particularly preferred tetrazine derivatives are those of g neral formula I wherein R^1 represents a straight- or branched-chain alkyl group containing from 1 to 6 carbon atoms, and more especially from 1 to 3 carbon atoms, unsubstituted or substituted by

More especially R^1 represents a methyl or 2-haloalkyl, e.g. 2-fluoroethyl or, preferably, 2-chloroethyl, group.

Preferably R^2 r presents a carbamoyl group or a monoalkylcarbamoyl, .g. methylcarbamoyl, or monoalkenylcarbamoyl group.

The present invention also includes salts of the compounds of general formula I wherein R^1 represents a hydrogen atom and R^2 is as hereinbefore defined, more especially alkali metal, e.g. sodium, salts, and whenever the context so permits reference to the compounds of general formula I in this specification is meant to include reference to the said salts. The salts are particularly useful as intermediates.

According to a feature of the present invention, th compounds of general formula I, wherein R^2 is as hereinbefore defined and R^1 is other than hydrog n, are prepared by the reaction of a compound of th general formula:–



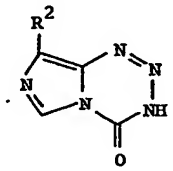
II

(wherein R^2 is as hereinbefore defined) with an isocyanate of the general formula:–

R^3NCO III

wherein R^3 represents an alkyl, alkenyl or alkynyl group, optionally substituted by one to three substituents selected from halogen atoms, alkoxy, alkylthio, alkylsulphinyl and alkylsulphonyl groups and optionally substituted phenyl groups, or represents a cycloalkyl group, within the definition of R^1 hereinbefore recited. The reaction may be effected in the absence or presence of an anhydrous organic solvent, for example a chlorinated alkane, e.g. dichloromethane, or ethyl acetate, acetonitrile, *N*-methylpyrrolid-2-one or, preferably, hexamethylphosphoramide, at a temperature between 0° and 70°C, e.g. at the ambient temperature. The reaction may be continued for up to 30 days. Light should preferably be excluded from the reaction mixture.

According to a further feature of the present invention, the compounds of general formula I, wherein R^2 is as hereinbefore defined and R^1 is other than hydrogen, are prepared by the reaction of a compound (within general formula I) of the general formula:–



IV

(wherein R^2 is as hereinbefore defined) or an alkali metal, e.g. sodium, salt thereof with a compound of the general formula:–

R^3X V

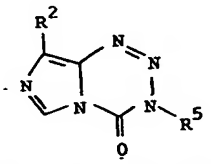
wherein R^3 is as hereinbefore defined, and X represents th acid residue of a reactive ester, for example a halogen (e.g. chlorine) atom, or a sulphuric or sulphonic ester residue, e.g. a methoxysulphonyloxy, methanesulphonyloxy, or toluene-*p*-sulphonyloxy

represents a haloalkyl, haloalkenyl or haloalkynyl group, the acid residue of a reactive ester represented by X will be selected from those known to be not less reactiv than the halogen atom substituent in R^3. When X in a compound of gen ral formula V represents a halogen atom, an alkali metal salt of the compound of general formula IV is preferably used and wherein X in a compound of general formula V represents a halogen atom and R^3 is a haloalkyl, haloalkenyl or haloalkynyl group wherein the halogen atom is the same as that represented by X, an excess of the dihalo compound of general formula V is preferably used. The reaction of a compound of general formula IV or alkali metal salt thereof with a compound of general formula V, wherein R^3 and X are as hereinbefore defined, may be carried out in a suitable anhydrous inert organic solvent, for example dichloromethane, acetonitrile or *N*-methylpyrrolid-2-one or mixtures thereof, at a temperature of from 0°C to 120°C and, when a compound of general formula IV is used, in the presence of an acid-binding agent, for example an alkali metal, e.g. sodium or potassium, carbonate or bicarbonate.

As a further feature of the invention, compounds of general formula IV (i.e. compounds of general formula I wherein R^1 represents a hydrogen atom and R^2 is as hereinbefore defined) or alkali metal salts thereof are prepared by the reaction of a compound of general formula II with a compound of the general formula:–

$$R^4NCO \qquad VI$$

wherein R^4 represents an alkali metal (e.g. sodium) atom or a protecting group such as a benzyl or *p*-methoxybenzyl group, followed, when R^4 represents a protecting group, by the replacement of the protecting group by a hydrogen atom in the compound thus obtained of the general formula:–



VII

wherein R^2 is as hereinbefore defined, and R^5 represents a protecting group such as a benzyl or *p*-methoxybenzyl group, by methods known *per se*. Reaction of a compound of general formula II with a compound of general formula VI wherein R^4 represents a protecting group may be effected as hereinbefore described for the reaction of a compound of general formula II with a compound of general formula III. Reaction of a compound of general formula II with a compound of general formula VI, wherein R^4 represents an alkali metal atom, may be effected in a suitable inert organic solvent, e.g. ethanol, acetonitrile or *N*-methylpyrrolidone, optionally in the presence of an acid, at a temperature of from 0° to 120°C. The group R^5 of compounds of g neral formula VII, wherein R^5 is as hereinbefore defined, may be replaced by a hydrogen atom by methods known *per se* to give a compound of general formula IV.

Compounds of general formula II may be prepared by the application or adaptation of methods known *per se*, for example methods described by Shealy J. Org. Chem. (1961), *26*, 2396.

Compounds of general formulae III, V and VI may be prepared by the application or adaptation of methods known *per se*.

By the term 'methods known *per se*' as used in the pres nt specification is meant methods heretofore used or described in the literature.

The new tetrazine derivatives of general formula I possess valuable antineoplastic activity, for example against carcinomas, melanomas, sarcomas, lymphomas and leukaemias. They have proved particularly active in mice at daily doses between 0.5 and 16 mg/kg animal body weight, administered intraperitoneally, against TLX(S) lymphoma according to the procedure of Gescher *et al*, Biochem. Pharmacol. (1981), *30*, 89, and ADJ/PC6A and M5076 (reticulum cell sarcoma). Against leukaemia L1210, grafted intraperitoneally, intracerebrally and intravenously, and P388, according to the procedure described in "Methods of Development of New Anticancer Drugs" (NCI Monograph 45, March 1977, pages 147-149, National Cancer Institue, Bethesda, United States), the compounds were active both intraperitoneally and orally at doses of between 2.5 and 10 mg/kg animal body weight. Inhibition of both primary tumour and metastasis was obtained against the Lewis lung carcinoma by similar dosage regimes. Against the B16 melanoma and C38 tumour in mice (NCI Monograph 45, *op cit*.) the compounds were active intraperitoneally at doses of between 6.25 and 25 mg/kg animal body weight.

The tetrazine derivatives also possess valuable immunomodulatory activity and are of use in the treatment of organ grafts and skin grafts and in the treatment of immunological diseases.

Important individual compounds of general formula I include the following:–

8-carbamoyl-3-methyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one A,

8-carbamoyl-3-n-propyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one B,

8-carbamoyl-3-(2-chloroethyl)-[3*H*]-imidazo-[5,1-d]-1,2,3,5-tetrazin-4-one C,

3-(2-chloroethyl)-8-methylcarbamoyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one D,

8-carbamoyl-3-(3-chloropropyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one E,

8-carbamoyl-3-(2,3-dichloropropyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one F,

3-allyl-8-carbamoyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one G,

3-(2-chloroethyl)-8-dimethylcarbamoyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one H,

3-(2-bromoethyl)-8-carbamoyl-[3*H*]-imidazo-[5,1-d]-1,2,3,5-tetrazin-4-one I,

3-benzyl-8-carbamoyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one J,

8-carbamoyl-3-(2-methoxyethyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one K,

8-carbamoyl-3-cyclohexyl-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-on L,

and 8-carbamoyl-3-(*p*-methoxybenzyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one. M

Compounds A and D, and especially C, are of par-

The letters A to M are allocated to the compounds for easy reference later in the specification.

The following Examples illustrate the preparation of compunds of general formula I according to the present invention, and the Reference Example thereafter illustrates the preparation of intermediates.

EXAMPLE 1

Compound A

4[5]-Diazoimidazole-5[4]-carboxamide (500 mg) was suspended in methyl isocyanate (3.0 ml) and stirred in the dark, at ambient temperature, for 21 days. The reaction mixture was then diluted with anhydrous diethyl ether and filtered. The residue was washed quickly with anhydrous methanol, then with anhydrous diethyl ether, and dried in air, in the dark, at ambient temperature, to give 8 - carbamoyl - 3 - methyl - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one, in the form of a light brown microcrystalline solid (198 mg), m.p. 210°C (with effervescence and darkening from 160° to 210°C). [Elemental analysys:– found C,36.8; H,3.10; 44.2%; $C_6H_6N_6O_2$ requires: C,37.1; H,3.09; N,43.3%].

EXAMPLE 2

Compound B

4[5] - Diazoimidazole - 5[4] - carboxamide (300 mg) was suspended in anhydrous dichloromethane (10 ml) and treated with an excess of n-propyl isocyanate. The reaction mixture was then stirred in the dark, at ambient temperature, for 30 days. The reaction mixture was then filtered and the residue was washed quickly with anhydrous diethyl ether, and dried in air, in the dark, at ambient temperature, to give 8 - carbamoyl - 3 - n - propyl - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one (102 mg), in the form of a pale pink powder, m.p. 167°C (with effervescence). [Elemental analysis:– found: C,43.4; H,4.57; N,38.0%; $C_8H_{10}N_6O_2$ requires: C,43.2; H,4.53; N,37.8%].

EXAMPLE 3

Compound C

4[5] - Diazoimidazole - 5[4] - carboxamide (300 mg) was suspended in anhydrous dichloromethane 10 ml) and 2 - chloroethyl isocyanate (1.0 ml) was added. The reaction mixture was then stirred in the dark, at ambient temperature, for 30 days. The cream-coloured suspension thus obtained was filtered and the residue was washed quickly with anhydrous diethyl ether and dried in air, in the dark, to give 8 - carbamoyl - 3 - (2 - chloroethyl) - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one (483 mg), in the form of a cream-coloured powder, m.p. 158°C (with vigorous decomposition). [Elemental analysis:– found: C,34.7; H,3.01; N,34.9%; $C_7H_7ClN_6O_2$ requires: C,34.7; H,2.91; N,34.7%].

Repetition of the above procedure has also given 8 - carbamoyl - 3 - (2 - chloroethyl) - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one in another polymorphic form, m.p. 164-165°C (with decomposition).

EXAMPLE 4

Compound A

A suspension of 4[5] - diazoimidazole - 5[4] - carboxamide (1.37 g) in ethyl acetate (20 ml) was treated with methyl isocyanate (7.0 g) and was stirred in a closed vessel in the dark at room temperature for 3 weeks. The resulting solid was filtered off and washed with diethyl ether to give 8 - carbamoyl - 3 - methyl - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one (1.9 g), in the form of a cream-coloured solid, m.p. 212°C (with effervescence).

This material was recrystallised from three different solvent systems to give three different products, each of which had a slightly different IR spectrum. The three products were probably all polymorphs of 8 - carbamoyl - 3 - methyl - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one.

(i) Colourless needles were obtained from a 3:1 v/v mixture of acetone and water, ν_{max} 3410, 3205, 1758, 1730 and 1678 cm^{-1}, m.p. 212°C (with effervescence).

(ii) White microcrystals were obtained from a 1:3 v/v mixture of acetone and water, ν_{max} 3430, 3200, 1740 and 1675 cm^{-1}, m.p. 210°C (with effervescence).

(iii) A granular solid was obtained from hot water, ν_{max}3450, 3380, 3200, 1742, 1688 and 1640 cm^{-1}, m.p. 215°C (with effervescence) (darkening from 200°C).

EXAMPLE 5

Compound B

A suspension of 4[5] - diazoimidazole - 5[4] - carboxamide (1.37 g) in acetonitrile (20 ml) was treated with n-propyl isocyanate (6.5 g) and was stirred in a closed vessel in the dark at room temperature for 3 weeks. The resulting pink solid was filtered off, washed with diethyl ether, and recrystallised from a mixture of water and acetone (1:4 v/v), to give 8 - carbamoyl - 3 - n- propyl - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one (1.6 g), m.p. 170-172°C (with effervescence). By concentration of the recrystallisation mother liquor there was obtained a further quantity (0.2 g) of the same product.

EXAMPLE 6

Compound C

A suspension of 4[5] - diazoimidazole - 5[4] - carboxamide (1.0 g) in ethyl acetate (30 ml) was treated with 2-chloroethyl isocyanate (3.3 ml) and the mixture was stirred in the dark, at ambient temperature, for 6 days. The reaction mixture was then diluted with diethyl ether and the resulting solid was filtered off, to give 8 - carbamoyl - 3 - (2 - chloroethyl) - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one (1.6 g) in the form of a colourless solid, m.p. 164-165°C (with decomposition). [Elemental analysis:– found: C,34.5; H,2.88; N,34.5; Cl,14.6%; $C_7H_7ClN_6O_2$ requires C,34.65; H,2.91; N,34.65; Cl,14.61%].

EXAMPLE 7

Compound C

A suspension of 4[5] - diazoimidazole - 5[4] - carboxamide (5.0 g) in a mixture of dichloromethane (158 ml) and *N* - methylpyrrolid - 2 - one (8.3 ml) was treated with 2-chloroethyl isocyanate (16.7 ml) and the mixture was stirred in the dark at ambient temperature for 14 days. The reaction mixture was then diluted with anhydrous diethyl ether and the resulting solid was filtered off and washed with diethyl ether, to give 8 - carbamoyl - 3 - (2 - chloroethyl) - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one (6.3 g), in the form of a purple-tinged solid, m.p. 164-165°C (with decomposition). [Elemental

analysis:– found: C,34.7; H,2.95; N,34.5; Cl,14.4%; $C_7H_7ClN_6O_2$ requires: C,34.65; H,2.91; N,34.65; Cl,14.61%].

EXAMPLE 8

Compound C

A suspension of 4[5] - diazoimidazole - 5[4] - carboxamide (145 g) in ethyl acetate (2175 ml) was treated with 2-chloroethyl isocyanate (478.5 ml) and stirred at 30°C, with the eclusion of light, for 2 days. The mixture was then filtered to give 8 - carbamoyl - 3 - (2 - chloroethyl) - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one (250 g), in the form of a peach-coloured solid, m.p. 166°C.

EXAMPLE 9

Compound A

A stirred suspension of 4[5] - diazoimidazole - 5[4] - carboxamide (2.2 g) in a mixture of dichloromethane (70 ml) and *N* - methylpyrrolid - 2 - one (3.5 ml) was treated with methyl isocyanate (7.0 ml) and stirred at ambient temperature for 4 weeks. The mixture was diluted with diethyl ether and the resulting solid was filtered off, to give 8 - carbamoyl - 3 - methyl - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one (2.38 g), in the form of a pale purple solid, m.p. 202-203°C (with decomposition). [Elemental analysis:– found: C,36.8; H,2.94; N,43.1%; $C_6H_6N_6O_2$ requires: C,37.11; H,3.14; N,43.3%].

A polymorphic form of 8 - carbamoyl - 3 - methyl - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one was obtained by dissolving it in acetanitrile, filtering, concentration of the filtrate to dryness, and trituration of the resulting residue with diethyl ether. This material was in the form of an orange-tinged solid, m.p. about 200°C (with decomposition). [Elemental analysis: C,37.4; H,3.26; N,43.5%]. Its NMR spectrum in dimethylsulphoxide - D_6 was identical to that of the abovementioned pale purple solid, but its IR spectrum (KBr disc) showed some differences.

EXAMPLE 10

Compound D

A stirred solution of sodium nitrite (0.64 g) in water (4.6 ml) was cooled to 5°-10°C and treated dropwise at that temperature with a solution of 5 - amino - 4 - methylcarbamoylimidazole (1.00 g) in aqueous acetic acid (1M; 14.3 ml) during 5 minutes. Stirring was continued at 5°-10°C for 5 minutes. The dark red solution was then extracted with ethyl acetate (4 x 35 ml) and the combined extracts were dried over magnesium sulphate. The resulting solution contained crude 4[5] - diazo - 5[4] - methylcarbamoylimidazole, which was unstable and was used immediately for the next stage without further purification.

The solution of 4[5] - diazo - 5[4] - methylcarbamoylimidazole in ethyl acetate, prepared as described abov , was treated with 2-chloroethyl isosyanate (4.3 ml) and was allowed to stand in the dark for 1 day. Th solution was then evaporated at 40°C/10 mm Hg and the residue was triturated with petroleum eth r (b.p. 40°-60°C) to give an orange gum (4.23 g). This gum was treated with ethyl acetate (50 ml) and filtered, and the filtrate was evaporated at 40°C/10 mm Hg to give an orange gum (2.94 g). This gum was purified by m dium pressure column chromatography on silica gel, luting with a give 3 - (2 - chloroethyl) - 8 - methylcarbamoyl - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one (0.81 g), in the form of a purple solid, m.p. 120-122°C (with decomposition). [Elemental analysis:– found: C,37.3; H,3.58; N,31.9%; $C_8H_9ClN_6O_2$ requir s: C,37.4; H,3.53; N,32.7%].

EXAMPLE 11

Compound E

A suspension of 4[5] - diazoimidazole - 5[4] - carboxamide (1.0 g) in ethyl acetate (50 ml; dried over anhydrous potassium carbonate) was treated with 3-chloropropyl isocyanate (4.86 g) and the mixture was stirred at ambient temperature for 3 days. The reaction mixture was then diluted with anhydrous diethyl ether and the resulting solid was filtered off, and washed with anhydrous diethyl ether, to give 8 - carbamoyl - 3 - (3 - chloropropyl) - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one (1.05 g) in the form of a pink solid, m.p. 153-154°C (with decomposition). [Elemental analysis:– found: C,37.1; H,3.42; N,32.7; Cl,13.8%; $C_8H_9ClN_6O_2$ requires: C,37.4; H,3.53; N,32.8; Cl,13.8%].

EXAMPLE 12

Compound F

By proceeding in a manner similar to that described hereinbefore in Example 11 but replacing the 3-chloropropyl isocyanate used as a starting material by the appropriate quantity of 2,3 - dichloropropyl isocyanate, there was prepared 8 - carbamoyl - 3 - (2,3 - dichloropropyl) - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one, in the form of an off-white solid, m.p. 153-155°C (with decomposition). [Elemental analysis:– found: C,32.7; H,2.51; N,28.7; Cl,24.1%; $C_8H_8Cl_2N_6O_2$ requires C,33.0; H,2.77; N,28.9; Cl,24.4%].

EXAMPLE 13

Compound G

Stirred allyl isocyanate (4.5 ml, redistilled immediately before use) was treated with 4[5] - diazoimidazole - 5[4] - carboxamide (1.0 g) and then with hexamethylphosphoramide (20 ml). The mixture was stirred at ambient temperature in the dark for 18 hours and then it was diluted with anhydrous diethyl ether and filtered. The resulting colourless solid was washed with anhydrous diethyl ether, to give 3 - allyl - 8 - carbamoyl - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one (1.6 g), in the form of a colourless solid, m.p. 149-150°C [ν_{max} (KBr disc): 1730, 1675 cm^{-1}; NMR in DMSO-d_6: singlets at 8.75, 7.67 and 7.60δ; double double triplet at 6.02 δ (J=5.5, 8, 10Hz), double doublet at 5.35 δ (J=1.5, 8 Hz) and 5.20 δ (J=1.5, 10 Hz) and doublet at 4.88 δ (J=5.5)].

EXAMPLE 14

Compound H

A solution of 4[5] - diazo - 5[4] - dimethylcarbamoylimidazole (1.59 g; prepared as described in Referenc Example 1 hereaft r) in dry ethyl acetate (57 ml) was treated with 2-chloroethyl isocyanate (6.36 g) and stirred at room temperature in the dark for 24 hours. The solution was then evaporat d *in vacuo* at 35°C, finally at 0.1 mm Hg to remove the excess of 2-chloroethyl isocyanate. The residual liquid was purified by medium pressure column chromatography of silica gel, eluting with a mixture

(2 - chloroethyl) - 8 - dimethylcarbamoyl - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one (0.82 g), in the form of colourless crystals, m.p. 114-116°C. [Elemental analysis:– found: C,39.7; H,3.95; N,30.8%; $C_9H_{11}ClN_6O_2$ requires: C,39.9; H,4.10; N,31.0%].

EXAMPLE 15

Compound I

A stirred suspension of 4[5] - diazoimidazole - 5[4] - carboxamide (1.0 g) in hexamethylphosphoramide (4 ml) was treated with 2-bromoethyl isocyanate (4.5 ml) and the mixture was stirred in the dark, at ambient temperature, for 2 days. The reaction mixture was then diluted with anhydrous diethyl ether and the resulting solid was filtered off, and washed with anhydrous diethyl ether, to give 3 - (2 - bromoethyl) - 8 - carbamoyl - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one (1.17 g), in the form of a colourless solid, m.p. 156-157°C (with decomposition). [Elemental analysis:– found: C,29.5; N,2.36; N,29.1; Br,27.3%; $C_7H_7BrN_6O_2$ requires: C,29.3; H,2.46; N,29.3; Br,27.8%].

EXAMPLE 16

Compound J

By proceeding in a manner similar to that described hereinbefore in Example 15 but replacing the 2-bromoethyl isocyanate used as a starting material by the appropriate quantity of benzyl isocyanate, there was prepared 3 - benzyl - 8 - carbamoyl - [3*H*] - imidazo - [5,1-d] - 1,2,3,5 - tetrazin - 4 - one (0.83 g), in the form of a buff-coloured solid, m.p. 176-177°C (with decomposition). [Elemental analysis:– found: C,53.6; H,3.66; N,31.0%; $C_{12}H_{10}N_6O_2$ requires: C,53.3; H,3.73; N,31.1%].

EXAMPLE 17

Compound K

A suspension of 4[5] - diazoimidazole - 5[4] - carboxamide (0.3 g) in acetonitrile (5 ml) was treated with 2-methoxyethyl isocyanate (0.5 g) and the mixture was stirred at between 45° and 47°C in the dark for 24 hours. The resulting solid was filtered off and washed with diethyl ether, to give crude 8 - carbamoyl - 3 - (2 - methoxyethyl) - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one (0.45 g), m.p. 145-147°C (with decomposition).

The product was purified by rescrystallisation from aqueous acetone to give pink rosettes, or from aqueous dimethylsulphoxide to give colourless needles, m.p. 164-165°C (with decomposition). [Elemental analysis: C,40.4; H,4.20; N,35.2%; $C_8H_{10}N_6O_3$ requires: C,40.34; H,4.20; N,35.2%].

EXAMPLE 18

Compound L

A suspension of 4[5] - diazoimidazole - 5[4] - carboxamide (0.30 g) in acetonitrile (10 ml) was treated with cyclohexyl isocyanate (1.0 g) and the mixture was stirred at 60°C in th dark for 3 days. The resulting solid was filtered off and washed with a mixture of ethanol and 0.880 aqueous ammonia (100:0.5 v/v; 20 ml) for one minute, to give 8-carbamoyl - 3 - cyclohexyl - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - on (0.015 g), m.p. 196°C (with effervescence).

EXAMPLE 19

Compound J

boxamide (0.4 g) in acet nitrile (10 ml) was treated with benzyl isocynate (0.6 g) and the mixture was stirred at 60°C in the dark overnight. The reacti n mixture was th n cooled and filtered, to give 3 - benzyl - 8 - carbamoyl - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one (0.75 g), in the form of a pale pink solid, m.p. 187-188°C (with effervescence).

EXAMPLE 20

Compound M

A suspension of 4[5] - diazoimidazole - 5[4] - carboxamide (0.1 g) and *p*-methoxybenzyl isocyanate (0.4 g) in acetonitrile (5 ml) was stirred at 60°C in th dark for 4 hours. The resulting pale pink solid was filtered off, and washed repeatedly with cold diethyl ether, to give 8 - carbamoyl - 3 - (*p*-methoxybenzyl) - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one (0.23 g), m.p. 180-182°C (with effervescence).

EXAMPLE 21

By proceeding in a similar manner to the foregoing Examples, there was prepared 8 - (N - allylcarbamoyl) - 3 - (2 - chloroethyl) - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one [I.R. 1750 cm^{-1}; NMR (in DMSO-d_6): multiplets 3.96, 5.06 and 5.84 ppm: triplets 4.60 and 6.2 ppm: singlet 8.78 ppm], from 5 - amino - 4 - allylcarbamoylimidazole via 5 - diazo - 4 - allylcarbamoylimidazole.

The 5 - amino - 4 - allylcarbamoylimidazole was prepared from 5 - nitro - 4 - allylcarbamoylimidazole (m.p. 218-220°C) by reduction by means of titanous chloride.

REFERENCE EXAMPLE

(i) an intimate mixture of 5 - nitroimidazole - 4 - carboxylic acid (2.0 g) and phosphorus pentachloride (2.67 g) was stirred and heated in an oil bath at 120°C for 1 hour. The resulting yellow slurry was evaporated at 60°C/0.1 mm Hg for 30 minutes, to give 1,6 - dinitro - 5*H*,10*H* - diimidazo[1,5-a:1',5'-d] pyrazine - 5,10 - dione (1.90 g) in the form of a yell w solid, m.p. 249-251°C (with decomposition). [ν_{max} (KBr disc) 1750 cm^{-1}; m/e 278 (M^+)].

Windaus, Ber., 1923, *56*, 684 and Gireva, Chem. Abs. *59*, 1622e, using the same method, describe their products as "5 - nitroimidazole - 4 - carbonyl chloride".

(ii) Aqueous dimethylamine solution (25% w/v; 60 ml) was cooled to between 0° and 5°C and treated portionwise, with stirring, with 1,6 - dinitro - 5H,10*H* - diimidazo[1,5-a:1',5' - d] pyrazine - 5,10 - dione (6.0 g) in that temperature range. The resulting deep purple solution was stirred for 2 hours. The solution was evaporated at 50°C/10 mm Hg and then acidified by treatment with concentrated hydrochloric acid, to give an orange solution. This solution was extracted with ethyl acetate (7 × 200 ml), and the combined extracts were dri d over magnesium sulphate, and evaporated, to give a yellow solid (6.6 g). This solid was triturated with toluene (50 ml) and then recrystallised from ethyl acetate, to give 5[4] - nitro - 4[5] - dimethylcarbamoylimidazole (2.53 g), in the form of yellow crystals, m.p. 193-195°C. [Elemental analysis:– found: C,38.9; H,30.4%; $C_6H_8N_4O_3$ requires: C, 39.1; H,4.38; N,30.4%].

(iii) A solution of 5[4] - nitro - 4[5] - dimethylcarbamoylimidazole (1.62 g) in dry dimethylformamide

shaken under hydrogen at atmospheric pressure and room temperature. After 3 hours, hydrogen absorption was complete (710 ml). The mixture was treated with charcoal and filtered through diatomaceous earth. The dark brown filtrate was evaporated at 50°C/0.1 mm Hg and the resulting residue was triturated with diethyl ether to give crude 5[4] - amino - 4[5] - dimethylcarbamoylimidazole (1.75 g), in the form of a dark brown crystalline solid, m.p. 179-181°C [ν_{max} (KBR disc) 1595 cm^{-1}; NMr in DMSO-d_6: singlets at 3.2 and 7.0δ], which was still contaminated with colloidal platinum and which was used in the next stage without further purification.

(iv) A stirred solution of sodium nitrite (0.79 g) in water (5.7 ml) was cooled to between 5° and 10°C and treated, dropwise, within this temperature range, with a solution of 5[4] - amino - 4[5] - dimethylcarbamoylimidazole (1.75 g) in aqueous acetic acid (1M; 17.6 ml) during 5 minutes. The resulting solution was extracted with ethyl acetate (4 × 40 ml), the combined extracts were dried over magnesium sulphate and evaporated at 30°C/10 mm Hg, to give 4[5] - diazo - 5[4] - dimethylcarbamoylimidazole (1.59 g), in the form of orange crystals, m.p. 101-103°C (with decomposition) [Elemental analysis:– found: C,42.6; H,4.17; N,41.4%; $C_6H_7N_5O$ requires: C,43.6; H,4.27; N,42.4%].

The present invention includes within its scope pharmaceutical compositions which comprise, as active ingredient, at least one tetrazine derivative of general formula I, together with a pharmaceutical carrier or coating. In clinical practice the compounds of general formula I will normally be administrated orally, rectally, vaginally or parenterally, e.g. intravenously or intraperitoneally.

Methods of presentation of pharmaceutically active compounds are well known in the art and a suitable vehicle may be determined by the physician or pharmacist, depending upon such factors as the effect sought, the size, age, sex and condition of the patient and on the properties of the active compound. The compositions may also contain, as is usual in the art, such materials as solid or liquid diluents, wetting agents, preservatives, flavouring and colouring agents and the like.

Solid compositions for oral administration include compressed tablets, pills, dispersible powders, and granules. In such solid compositions one or more of the active compounds is, or are, admixed with at least one inert diluent such as calcium carbonate, potato starch, alginic acid, or lactose. The compositions may also comprise, as is normal practice, additional substances other than inert diluents, e.g. lubricating agents, such as magnesium stearate. Liquid compositions for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups and elixirs containing inert diluents commonly used in the art, such as water and liquid paraffin. Besides inert diluents such compositions may also comprise adjuvants, such as wetting and suspending agents, .g. polyvinylpyrrolidone, and sweetening, flavouring, perfuming and preserving agents. The compositions according to the invention, for oral administration, also include capsules of absorbable material such as gelatin containing one or more of the active substances with or without the addition of diluents or excipients.

Solid compositions for vaginal administration include pessaries formulated in manner known *per se* and containing one or more of the active compounds.

Solid compositions for rectal administration include suppositories formulated in manner known *per se* and containing one or more of the active compounds.

Preparation according to the invention for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of non-aqueous solvents or suspending media are polyethylene glycol, dimethyl sulphoxide, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. These compositions may also include adjuvants such as preserving, wetting, emulsifying and dispersing agents. They may be sterilised, for example, by filtration through a bacteria-retaining filter, by incorporation of sterilising agents in the compositions, or by irradiation. They may also be manufactured in the form of steril solid compositions, which can be dissolved in sterile water or some other sterile injectable medium immediately before use.

The percentage of active ingredient in the compositions of the invention may be varied, it being necessary that it should constitute a proportion such that a suitable dosage for the therapeutic effect desired shall be obtained. Obviously several unit dosage forms may be administered at about the same time. In general, the preparations should normally contain at least 0.025% by weight of active substance when required for administration by injection; for oral administration the preparation will normally contain at least 0.1% by weight of active substance. The dos employed depends upon the desired therapeutic effect, the route of administration and the duration of the treatment.

The tetrazine derivatives of general formula I are useful in the treatment of malignant neoplasms, for example carcinomas, melanomas, sarcomas, lymphomas and leukaemias, at doses which are generally between 0.1 to 200, preferably between 1 and 20, mg/kg body weight per day.

The following Composition Examples illustrate pharmaceutical compositions according to the present invention.

COMPOSITION EXAMPLE 1

A solution suitable for parenteral administration was prepared from the following ingredients:–

8-Carbamoyl-3-(2-chloroethyl)-[3*H*]-imidazo[5,1-d]-1,2,3,5-tetrazin-4-one	1.0 g
Dimethyl sulphoxide	10 ml
Arachis oil	90 ml

by dissolving the 8-carbamoyl - 3 - (2 - chloroethyl) - [3*H*] - imidazo [5,1-d] - 1,2,3,5 - tetrazin - 4 - one in the dimethyl sulphoxide and adding the arachis oil. The resulting solution was divided, under aseptic conditions, into ampoules at an amount of 10 ml per ampoule. The ampoules were sealed, to give 10 ampoules each containing 100 mg of 8 - carbamoyl - 3 - (2 - chloroethyl) - [3*H*] - imidazo [5,1-d] - 1,2,3,5 - tetrazin - 4 - one

Similar ampoules containing solutions suitable for parenteral administration may be prepared by proceeding in a similar manner but replacing the 8 - carbamoyl - 3 - (2 - chloro thyl) - [3*H*] - imidazo - [5,1-d] - 1,2,3,5 - tetrazin - 4 - one by another compound of general formula I.

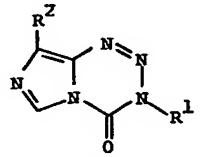
COMPOSITION EXAMPLE 2

Capsules suitable for oral administration were prepared by placing 8 - carbamoyl - 3 - (2 - chloroethyl) - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one into gelatin shells of number 2 size at a rate of 10 mg per capsule.

Similar capsules may be prepared by using another compound of general formula I or any other conveniently sized capsule shells.

CLAIMS

1. [3*H*] - Imidazo[5, 1-d] - 1, 2, 3, 5 - tetrazin - 4 - one derivatives of the general formula:—



I

wherein R^1 represents a hydrogen atom, or a straight- or branched-chain alkyl, alkenyl or alkynyl group containing up to 6 carbon atoms, each such group being unsubstituted or substituted by from one to three substituents selected from halogen atoms, straight- or branched-chain alkoxy, alkylthio, alkylsulphinyl and alkylsulphonyl groups containing up to 4 carbon atoms, and optionally substituted phenyl groups, or R^1 represents a cycloalkyl group containing from 3 to 8 carbon atoms, and R^2 represents a carbamoyl group which may carry on the nitrogen atom one or two groups selected from straight-and branched-chain alkyl and alkenyl groups, each containing up to 4 carbon atoms, and cycloalkyl groups containing from 3 to 8 carbon atoms and—when R^1 represents hydrogen—alkali metal salts thereof.

2. Tetrazine derivatives according to claim 1 wherein R^1 represents an alkyl, alkenyl or alkynyl group substituted by one, two or three optionally substituted phenyl groups and the optional substituents on the phenyl radical(s) are selected from alkoxy and alkyl groups containing up to 4 carbon atoms, and the nitro group.

3. Tetrazine derivatives according to claim 1 wherein R^1 represents a straight- or branched-chain alkyl group containing from 1 to 6 carbon atoms optionally substituted by one or two halogen atoms or by an alkoxy group containing 1 to 4 carbon atoms or by a phenyl group optionally substituted by one or two alkoxy groups containing from 1 to 4 carbon atoms, or R^1 represents an alkenyl group containing 2 to 6 carbon atoms or a cyclohexyl group.

4. Tetrazine derivatives according to claim 3 wherein the halogen atom(s) is (or ar) chl rine, flu rine and/or bromine, the alk xy gr up(s) is (or are) methoxy, and the alkenyl group is allyl.

5. Tetrazine derivatives according to claim 1 wherein R^1 represents a straight- or branched-chain alkyl group containing from 1 to 6 carbon atoms, unsubstituted or substituted by a halogen atom.

6. Tetrazine d rivatives according to claim 5 wherein R^1 r presents an alkyl group containing 1 to 3 carbon atoms unsubstituted or substituted by a halogen atom.

7. Tetrazine derivatives according to claim 1 or 5 wherein R^1 represents a methyl or 2 - haloalkyl group.

8. Tetrazine derivatives according to claim 5, 6 or 7 in which the halogen atom on the alkyl group is chlorine or fluorine.

9. Tetrazine derivatives according to claim 1 wherein R^1 represents a 2 - fluoroethyl or 2 - chloroethyl group.

10. Tetrazine derivatives according to claim 1 wherein R^1 represents a benzyl or *p*-methoxybenzyl group.

11. Tetrazine derivatives according to any one of claims 1 to 10 wherein R^2 represents a carbamoyl group, a monoalkylcarbamoyl group containing up to 4 carbon atoms in the alkyl radical, or a monoalkenylcarbamoyl group containing up to 4 carbon atoms in the alkenyl radical.

12. Tetrazine derivatives according to claim 1 wherein R^1 represents a straight- or branched-chain alkyl, alkenyl or alkynyl group containing from 1 to 6 carbon atoms, each such group being unsubstituted or substituted by from one to three halogen atoms, and R^2 represents the carbamoyl group.

13. 8 - Carbamoyl - 3 - methyl - [3*H*] - imidazo[5, 1-d] - 1,2,3,5 - tetrazin - 4 - one.

14. 8 - Carbamoyl - 3 - n - propyl - [3*H*] - imidazo [5,1-d] - 1,2,3,5 - tetrazin - 4 - one.

15. 8 - Carbamoyl - 3 - (2 - chloroethyl) - [3*H*] - imidazo - [5,1-d] - 1,2,3,5 - tetrazin - 4 - one.

16. 3 - (2 - Chloroethyl) - 8 - methylcarbamoyl - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one.

17. 8 - Carbamoyl - 3 - (3 - chloropropyl) - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one.

18. 8 - Carbamoyl - 3 - (2,3 - dichloropropyl) - [3*H*] - imidazo[5, 1-d] - 1,2,3,5 - tetrazin - 4 - one.

19. 3 - Allyl - 8 - carbamoyl - [3*H*] - imidazo [5, 1-d] - 1,2,3,5 - tetrazin - 4 - one.

20. 3 - (2 - Chloroethyl) - 8 - dimethylcarbamoyl - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one.

21. 3 - (2 - Bromoethyl) - 8 - carbamoyl - [3*H*] - imidazo - [5, 1-d] - 1,2,3,5 - tetrazin - 4 - one.

22. 3 - Benzyl - 8 - carbamoyl - [3*H*] - imidazo [5, 1-d] - 1,2,3,5 - tetrazin - 4 - one.

23. 8 - Carbamoyl - 3 - (2 - methoxyethyl) - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one.

24. 8 - Carbamoyl - 3 - cyclohexyl - [3*H*] - imidazo [5,1-d] - 1,2,3,5 - tetrazin - 4 - one.

25. 8 - Carbamoyl - 3 - (*p* - methoxybenzyl) - [3*H*] - imidazo[5,1-d] - 1,2,3,5 - tetrazin - 4 - one.

26. 8 - (N-Allylcarbamoyl) - 3 - (2 - chloroethyl) - [3*H*] - imidazo [5, 1-d] - 1,2,3,5 - tetrazin - 4 - on .

27. A process for the preparation of a tetrazine derivative of the general formula depicted in claim 1, wherein R^1 and R^2 are as defined in claim 1 but excluding hydrogen from the definition of R^1, which comprises reacting a compound of the general f rmula:

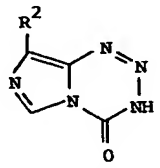
II

(wherein R^2 is as defined in claim 1) with an isocyanate of the general formula:–

$$R^3NCO \qquad III$$

wherein R^3 represents an alkyl, alkenyl or alkynyl group containing up to 6 carbon atoms, each such group being unsubstituted or substituted by one to three substituents selected from halogen atoms, alkoxy, alkylthio, alkylsulphinyl and alkylsulphonyl groups containing up to 4 carbon atoms, and optionally substituted phenyl groups, or R^3 represents a cycloalkyl group containing 3 to 8 carbon atoms.

28. A process for the preparation of a tetrazine derivative of the general formula depicted in claim 1, wherein R^1 and R^2 are as defined in claim 1 but excluding hydrogen from the definition of R^1, which comprises reacting a compound of the general formula:



IV

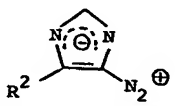
(wherein R^2 is as defined in claim 1) or an alkali metal salt thereof with a compound of the general formula:–

$$R^3X \qquad V$$

wherein R^3 is as defined in claim 27, and X represents the acid residue of a reactive ester.

29. A process according to claim 28 wherein X represents a halogen atom or a methoxysulphonyloxy, methanesulphonyloxy or toluene-*p*-sulphonyloxy group.

30. A process for the preparation of a tetrazine derivative of the general formula depicted in claim 1, wherein R^1 represents a hydrogen atom and R^2 is as defined in claim 1, or an alkali metal salt thereof which comprises reacting a compound of the general formula:

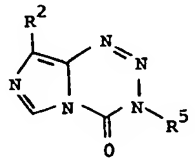


II

with a compound of the general formula:–

$$R^4NCO \qquad VI$$

wherein R^4 represents an alkali metal atom or a protecting group such as a benzyl or *p*-methoxybenzyl group, followed, when R^4 represents a prot cting group, by the replacement of the protecting group by a hydrogen atom in the compound thus obtain d of the general formula:–



VII

wherein R^2 is as defined in claim 1 and R^5 represents a prot cting group such as a benzyl or *p*-methoxybenzyl group, by methods known *per se*.

31. A process for the preparation of a tetrazine derivative of the general formula depicted in claim 1, wherein R^1 and R^2 are as defined in claim 1, or – when R^1 represents hydrogen – an alkali metal salt thereof substantially as hereinbefore described with especial reference to any of Examples 1 to 21.

32. Pharmaceutical compositions which comprise, as active ingredient, at least one tetrazine derivative as claimed in any one of claims 1 to 26 in association with a pharmaceutical carrier or coating.

33. Pharmaceutical compositions according to claim 32 substantially as hereinbefore described with especial reference to Composition Example 1 r 2.

34. Tetrazine derivatives of the general formula depicted in claim 1, wherein R^1 and R^2 are as defined in claim 1, for use in the treatment of malignant neoplasms such as carcinomas, melanomas, sarcomas, lymphomas and leukaemias.

35. Tetrazine derivatives of the general formula depicted in claim 1, wherein R^1 and R^2 are as defined in claim 1, for use in the treatment of organ grafts and skin grafts and in the treatment of immunological diseases.

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